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STUDIES IN THE GAMETOGENESIS OF FISHES—THE ROLE OF NUCLEOLI IN OOGENESIS

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INTRODUCTION

SINCE the discovery of the Nucleolus by Fontana in 1781[20] many workers have tried to throw light on its nature and functions, the common belief being that the nucleoli play an important role in the interaction between the nucleus and the cytoplasm. Again while some workers assert that the nucleoli are cast bodily into the cytoplasm where they fulfil their future destiny others deny the existence of any such process. In view of this conflicting state of affairs an effort has been made to investigate the role of nucleoli in the oogenesis of some fishes.

Part of the cost of this investigation has been met by a grant from the Scientific Research Committee, U.P., for which I express my thanks.

MATERIAL AND TECHNIQUE

The ovarian tissue of the fishes, *viz.*, *Notopterus chitala*, *Tetradon fluviatalis*, *Clarias batrachus*, *Rita rita*, *Ophiocephalus punctatus* and *Heteropneusteus fossilis* has been examined. The last two were kept alive in water in earthen pots and examined at convenience while the others were obtained fresh from local rivers and ponds. Bouin's Picro formol and Carnoy's alcohol-acetic methods were mainly used for fixation, while Mann's methyl

blue eosine and iron-alum hæmatoxylin were employed for staining. The Chrome Osmium techniques followed by iron-alum hæmatoxylin and Champy-Kull methods were also tried. Sudan IV was used to ascertain the relationship between nucleoli and fat formation. Pieces of fresh tissue were also teased out and mounted in physiological salt solution and examined as such.

OBSERVATIONS

The oogonia and very early oocytes contain relatively large nuclei each with a single nucleolus (Fig. 1). The conversion of the oogonium into the oocyte is characterised not only by the increase in the amount of cytoplasm but also in the number of nucleoli and I find instead of the single nucleolus of the oogonium a large number of nucleoli ranging from 4 to 8 (Fig. 2). In older oocytes the number increases further going up to 30 or more (Fig. 3). In spite of this obvious increase in numbers the actual division or fission of the nucleoli could not be observed, and it cannot be said at present if they arise *de novo* or are formed by the division of the pre-existing ones, though the enormous difference in the size of the biggest and the smallest ones is suggestive of the idea that there is no regular division and that probably new nucleoli arise by the fragmentation of the older ones. The nucleoli are mostly rounded or oval in shape. In the early oocytes they are irregularly distributed throughout the nuclear space but in the older ones they come to lie close beneath the nuclear membrane. The nucleoli are fixed well by Bouin and Carnoy's fixatives. With Osmic techniques they turn brown to black after long periods of osmication, say of a week or more. By this time the entire tissue assumes a blackish colour. The assumption of the black colour by the nucleoli along with the entire body of the cell after the prolonged osmication should not be taken to mean that these structures are fatty in nature, rather their fixation by Bouin, etc., indicates that they are formed of albuminous material.

The extrusion of the nucleoli from the nucleus into the surrounding cytoplasm commences in the early oocytes, but becomes prominent during the later stages of oogenesis when their number has increased very much. During this period a few structures similar to the nucleoli within the nucleus may be seen in the cytoplasm lying near the nuclear membrane (Figs. 3, 4 and 5). These have been identified as nucleolar extrusions owing to the following considerations:—

(1) In their colour reactions they correspond to those of the nucleoli still within the nucleus. The oogonial nucleus and the nucleoli of the early oocytes stain red with Mann's methyl blue eosine and are accordingly regarded as oxyphil. During the development of the oocyte a change occurs

and the nucleoli gradually lose their affinity for eosine. In the middle sized oocytes we find that the margin of the nucleolus takes the methyl blue colour while the central part still colours red with eosine. In the older oocytes and yolky eggs the entire body of every nucleolus colours blue (basophil reaction). The exuded nucleoli like those within the nuclear membrane are oxyphil in the very young oocytes but are basophil in the older ones.

(2) These structures are fixed nicely by Bouin and corrosive acetic fixatives, hence they are not likely to be mitochondria or Golgi bodies as these organelles are dissolved by the acid containing fixatives. Further as they are seen even in the very young oocytes when yolk formation has not begun, they cannot be regarded as yolk granules.

(3) Both in the preserved and the fresh material spherules of the same size and shape as the usual nucleoli are occasionally seen lying along the nuclear membrane and the cytoplasm (Figs. 4 and 5). I believe these are nucleoli actually in the process of extrusion and are on their way to be thrown out of the nucleus into the cytoplasm.

(4) In the oldest oocytes the number of nucleoli is found to be appreciably smaller as compared to that in the medium-sized ones. This decrease in the numbers is most probably due to a good many of the nucleoli having passed out into the cytoplasm.

Regarding the role of the nucleolar extrusions in the oocytes of fishes nothing definite is known. The extruded nucleoli are of various sizes, some being fairly big while others are much smaller. The emission is not confined to any one point but takes place on all sides of the nucleus. It is clearly discernible in young and medium-sized oocytes but as the yolk accumulates, the process becomes less frequent and difficult to observe. The extruded nucleoli travel outwards but do not appear to go far from the nuclear membrane. No vacuoles have been noted to form round the extruded nucleoli, and after their exit from the nucleus they gradually lose their power of staining and become indistinguishable from the surrounding cytoplasm. It is probable that they take no direct part in vitellogenesis though the enormous increase in their numbers at certain stages is suggestive of some function unknown at present.

As to how the nucleolar extrusions pass out of the nuclear membrane is not clear. In the fresh preparations as well as in those got by the majority of fixatives the nuclear membrane is smooth and regular, only rarely in Bouin and Carnoy preparations the outline of the nucleus appears irregular. This is obviously due to the contraction caused by the fixatives. However in none of the preparations could be found any constrictions or separation

of nucleolar pockets as described by Scharff[28] in *Trigla gurnardus* and by Eggert[8] in *Salarus flavo* umbrinus and *Gobius panizzne*. Occasionally some nucleoli during extrusion are found to be somewhat pointed (Fig. 5). It may be that they assume this shape to be able to pass out of the nuclear membrane.

DISCUSSION

It is now regarded as a well-established fact that the nucleolar extrusions do occur in many animals. Gatenby,[13] Hogben,[15] Nath[23, 24] and Ludford[20] have shown their occurrence in a number of Invertebrates. Prominent among the workers on the vertebrates in this line are Will[34], Henneguy,[17] Loyez,[18] Gajewaka[10] and Bhattacharya.[1]

Recently however Gall, J. G.,[9a] working on Lampbrush chromosomes from the Oocyte Nuclei of the Newt has shown his skepticism regarding the very occurrence of nucleolar extrusions into the cytoplasm as follows:—

" Reports appear from time to time that nucleoli can move bodily through the nuclear membrane into the cytoplasm where they presumably take part in yolk formation on other synthetic processes. Among the more recent accounts of this phenomenon may be mentioned that of Wittek (1952) in *Amphibia*, of Dodson (1953) in Squalus and Amphiuma, and of Chaudhry (1951) in Teleost fishes. Conclusions of this sort based on sectioned material must be accepted with caution. Many of my own sections of Newt oocytes show nucleoli in the cytoplasm or even half-way through the nuclear membrane. However, a further examination shows that most of these nucleoli are on one side of the oocyte and have been simply dragged to their present position by microtome blade." In my preparations nucleolar extrusions have been observed on all sides of the nucleus (Fig. 3) and I do not think the extrusion can be caused by the microtome blade, in hundreds of sections that have been studied. Such mechanical effect must be rare and cannot be a regular phenomenon.

It cannot be denied in the face of so much evidence that nucleolar extrusions do occur in certain animals.

The majority of workers on fish oogenesis have figured the nucleoli in the oocytes, but few seem to have studied their behaviour in detail. Wallace[33] with regard to this phenomenon in fishes says, " In all probability the membrane of the germinal vesicle is as Carnoy and Lebrun have stated imperforate so that no transmission of solid material but only osmosis can take place through it." Nath[22] while doubting the actual occurrence of nucleolar extrusions in fishes (*Rita* and *Ophiocephalus*) believes that there may be

some influence of the nucleus on the processes of vitellogenesis. His observations are as follows:—

"In course of oogenesis a large number of nucleoli appear, but no nucleolar buds are extruded into the cytoplasm and none have been described by any previous worker either in the frog or in the fish. But the enormous increase in the size of the nucleus and the multiplication of the nucleoli seem to indicate that the nucleus is controlling in some manner the process of vitellogenesis. It may be that some material which cannot be detected by the microscope passes from the nucleolus into the cytoplasm." However it may be pointed out that a few earlier workers, *e.g.*, Calderwood,[3] Scharff,[28] and Eggert[8] have noted the phenomenon of nucleolar extrusions in a few teleost fishes. Eggert[8] has found in *Salarius flabo-umbrinus* and other species of Gobidæ that the nuclear membrane is thrown into projections each one containing a nucleolus. According to him these projections constrict, get separated and wander away into the cytoplasm where they finally disappear. Though I find distinct nucleolar extrusions I have not noticed the separation of nuclear pockets as described by Eggert. Scharff[28] maintains that in *Trigla gurnardus* the nucleoli give rise to yolk spherules, while Calderwood[3] in the case of *Conger* thinks that, "There is probably some connection between these vesicles (nucleoli) and oil globules." Wallace[33] however contradicts the above observations of Scharff and Calderwood as he did not find anything comparable to these results in the teleosts he examined. Subramaniam and Aiyar[30] working on the oogenesis of a fish remark, "From our observations on *Gobius neilli* we believe that fat arises in the oocyte by osmosis of material through the nuclear membrane." In my preparations I do not find the formation of fat from the nucleoli either within the nucleus, or after their extrusion into the cytoplasm; nor did I notice any reduction in the size of the nucleoli with the growth of the oocyte.

Singh and Boyle[29] working on Stickleback have recorded their observations as follows:—

"A remarkable thing about the nucleoli of the oocytes of all ages is the fact that some of them are plastered on the inside of the nuclear membrane (Figs. 10 and 11, Pl. IV). After some of the fixatives employed the nuclear substance is slightly shrunken so that it is withdrawn from the nuclear membrane. When this occurs some of the nucleoli which had been applied to the nuclear membrane are drawn away. In this position threads can be seen extending from the nucleoli through the nuclear membrane. In the younger oocytes these threads pass through the nuclear membrane and extend out to the periphery of the cell (Figs. 14 and 15, Pl. V). As the cell gets

larger these threads break up into granules which at first are arranged in lines (Fig. 16, Pl. V). Further extrusion of the nuclear material goes on in the form of granules so that the nucleus is surrounded by a dense cloud of granules extending out into the cytoplasm. These appeared particularly clearly after Bouin fixative followed by staining in Mann's methyl blue eosine. These granules then move away from the nucleus and pass out in a band towards the periphery of the cell. At the periphery of the cell they swell up, become surrounded by a vacuole and transform into yolk (Fig. 17, Pl. V). As the yolk is formed the spheres enlarge and are found scattered throughout the whole cytoplasm of the egg. Some of the yolk bodies are irregular in shape. The process of the formation of yolk can be followed clearly after fixation in Bouin followed by staining in iron-alum hæmatoxylin or Mann's methyl blue eosine."

However I have to point out that in none of the fishes examined by me by the techniques employed by these workers I have ever seen anything comparable to the above observations. Rarely the nuclear membrane may be shrivelled a little owing to fixation and the nucleoli may be slightly separated from it (Fig. 6) but they have never been observed to leave a trail behind in the form of threads, nor have such threads been observed in the study of fresh oocytes. Most probably the threads described by these authors are artefacts.

Subramaniam and Aiyer[30] and Chaudhry[6] who have described in recent years the nucleolar extrusions in the oocytes of fishes, could not notice any threads of nuclear material being cast out into the cytoplasm and breaking up into granules. Further Chaudhry[6] could not confirm the origin of fat or albuminous yolk from nucleolar extrusions and is in agreement with my earlier observations[25, and 23] and the present findings that the nucleolar extrusions, if at all, play only some indirect part in the oogenesis and are not directly responsible for the formation of any well marked substance. Similarly Ludford[20] has proved the occurrence of nucleolar extrusions in Limnæa, but no definite substance was found to arise from them. Again Gresson[12] in mouse and Clement[5] in squirrel, find that these bodies take no discernible part in vitellogenesis. Recently Robertis and others[27a] have observed that, "In the teleosts, the oocytes have at a particular time in development, numerous nucleoli which adhere to the karyotheca and in more advanced stages, bodies with characteristics of the nucleoli are found not only within the nucleus, but also in the perinuclear cytoplasm." This is clearly in line with the finding that the nucleoli as such are extruded from the nucleus into the cytoplasm in the oocytes of teleosts.

Another question of some significance in connection with the extrusion of the nucleoli, is, as to how they are emitted from the nucleus into the cytoplasm. The majority of workers on oogenesis are silent on this point. The nucleus in fishes (at least in preserved material), appears to have a delicate membrane bounding it. Now if it were a firm layer, no solid granules could possibly escape out of it, unless it were perforated at certain places to allow of the exit of these bodies. However, I have not been able to observe in any of my slides any perforations in the nuclear membrane. This possibility has therefore to be discarded. The only other possible explanation of the question as to how these extrusions pass out of the nuclear membrane, is to imagine that the nuclear membrane during life-time is not solid but semi-solid or gelatinous and can allow the bodies to pass through it. When something is passing out the membrane gives way, but so soon as it has gone out the two ruptured ends meet one another and the membrane assumes its original appearance. If this be true, it will explain also the presence of a regular non-perforated membrane in the fixed material. Gardiner[11] describing the nucleolar extrusions in *Limulus* says, " There is no indication that the buds are constricted off as such, it seems rather that the condition is one in which the internal pressure of the nucleolus has risen to such a degree that some of its substance is forced out—a process comparable to the bursting of a bubble." Ludford holds somewhat similar views on this point. In his paper on Patella[19] he says, " During the process of differentiation into two parts there seems always to be an extrusion of oxyphil substance into the cytoplasm. This is shown specially strikingly where the nucleolus is lobulated and oxyphil fragments are breaking away from it and passing out through the nuclear membrane into the cytoplasm. The nuclear membrane does not appear to be damaged by this process which recalls the passage of leucocytes through the walls of the blood capillaries."

In the end it may not be out of place to point out that at present there exists a great difference of opinion among cytologists regarding the nature and function of the nucleolus. Some relate it with chromosome formation, a few think that it is composed of the waste products of the nuclear metabolism, while still others hold that it is used up in the manufacture of specific cell products. This has been proved only in a few animals where albuminous yolk has been found to be formed from nucleolar extrusions, but in many animals it has not been possible to ascribe any particular function to the nucleolus.

Summary

1. The definite occurrence of nucleolar extrusions from the nucleus into the cytoplasm has been observed in the oocytes of a number of fishes,

e.g., Notopterus, Tetradon, Ophiocephalus, Heteropneustes, Clarias and *Rita.*

2. No specific function of the extruded nucleoli could be discovered. The view of some workers that they form albuminous yolk could not be confirmed.

3. The nucleolar extrusions do not form fat in the oocytes of the fishes examined.

BIBLIOGRAPHY

1. BHATTACHARYA, D. R., 1925, "Les inclusions cytoplasmiques dans l'oogenese de certains Reptiles," *These Sciences de Paris.*
2. BRAMBELL, F. W. R., 1925, "The oogenesis of the Fowl," *Phil. Trans. Roy. Soc., London,* **214 B.**
3. CALDERWOOD, W., 1892, "A contribution to our knowledge of the Ovary and the Intraovarian egg in Teleosteans," *J. Mar. Biol. Asso. U.K.*, 2.
4. CONKLIN, E. G., 1910, "Effects of Centrifugal force upon the organisation and development of the eggs of Freshwater Pulmonates," *J. Expt. Zool.*, **9.**
5. CLEMENT, R., 1933, "On the Cytoplasmic Inclusions in the Oogenesis of *Sciurus palmarum,*" *Alld. Uni. Studies,* **10.**
6. CHAUDHRY, H. S., 1951, "Nucleolar Activity in the Oocytes of Some Marine Teleost Fishes," *J.R.M.S.*, **71.**
7. CUNNINGHAM, J. T., 1897, "On the Histology of the Ovary and the Ovarian Ova in Certain Marine Fishes," *Quart. J. Micr. Sci.*, **40.**

7 *a.* DODSON, E. O., "Nucleoli and the formation of yolk in the eggs of vertebrates," *J. R. Micr. Soc.*, **72**, 177–78.

8. EGGERT, B., 1929, "Entwicklung und Bau der Eier von *Salarias flaboumbrinus,*" *Zool. Anz.*, **83.**
9. ———, 1931, *Ibid., Z. wiss. Zool.*, **139.**

9 *a.* GALL, J. G., 1954, "Lampbrushrus chromosomes from oocyte nuclei of the Newt," *Journ. of Morph.*, **94** (2).

10. GAJEWAKA, H., 1917, "Ueber die morphologischen veranderungen des kern und Plasmmesubstanzen im verlaufe des wachstums der oocyten," *Archiv. fur Zellforschung,* **14.**
11. GARDINER, M. S., 1927, "Oogenesis in *Limulus polyphemus,* with special reference to the behaviour of the nucleolus," *Jour. Morph. and Physiol.*, **44.**
12. GRESSON, R. A., 1929, "Nucleolar phenomena during oogenesis in certain Tenthredinidæ," *Quart. Jour. Micr. Sci.*, **73.**
13. GATENBY, J. B., 1917–22, "The Cytoplasmic Inclusions of Germ Cells," *Ibid.*, **63**, **64** and **66.**

14. GATENBY, J. B. AND PAINTER, T. S., 1937, *The Microtomists,' Vade Mecum (Bolles Lee)*. Tenth edition, J. and A. Churchill, London.

15. HOGBEN, L. T., 1920, "Studies on Synapsis II. Parallel conjugation and the prophase comex in Periplaneta with especial reference to the pre-meiotic Telophase," *Proc. Roy. Soc.*, **91** B.

16. HIBBARD, H. AND PARAT, M., 1927, "Oogenesis in certain Teleosts with special reference to the Chodriome, Vacuome and yolk formation," *J. Anat. Phy.*, **61**.

17. HENNEGUY, L. F., 1893, "Le Corps de Balbiani chez Le vertebrate," *Journ. De l'anat.*

18. LOYEZ, M., 1902, "Les premiers stades du development du la vasicule germinative chez les Reptiles," *C. R. Acad. des Science de Paris, t* **133**, et *C. R. Assoc. des Anat. Montpellier.*

19. LUDFORD, R. J., 1921, "Contributions to the study of the oogenesis of Patella," *Jour. Roy. Micr. Soc.*, **41**.

20. ————, 1922, "The Morphology and Physiology of the Nucleolus. Part I. The Nucleolus in the Germ cell cycle of the Mollusc *Limnea stagnalis*," *Ibid.*

21. NAKAHARA, W., 1917, "On the Physiology of the Nucleoli as seen in the silk gland of certain insects," *Journ. Morph.*, **29**.

22. NATH, V. AND NANGIA, M. D., 1931, "A demonstration of the Vacuome and the Golgi apparatus as independent cytoplasmic components in the fresh eggs of Teleostean Fishes," *J. Morph. Physiol.*, **52**.

23. NATH, V. AND MEHTA, D. R., 1929, "Studies in the Origin of Yolk. III. Oogenesis of the firefly *Luciola gorhami*," *Quart. Journ. Micr. Sci.*, **73**.

24. NATH, V. AND MOHAN, P., 1929, "Studies in the Origin of Yolk. IV. Oogenesis of *Periplaneta americana*," *Journ. Morph. and Physiol.*, **48**.

25. NARAIN, D., 1930, "Cytoplasmic Inclusions in the Oogenesis of *Ophiocephalus punctatus*," *Z. Zellforsch.*, **11**.

26. ————, 1937, "Cytoplasmic Inclusions in the Oogenesis of *Sacchobranchus fossilis, Clarias batrachus* and *Anabas scandens*," *Ibid.*, **26**.

27. NATH, V. SINGH, B. AND BAKR, A., 1944, "Oogenesis of *Sacchobranchus fossilis* and *Cirrhina mrigala*, etc., with especial reference to nucleolar extrusions," *Proc. Nat. Inst. Sci., India*, **10**.

27 *a*. ROBERTIS, E. D., NOWINSKI, W. W. AND SAES, F. A., 1954, *General Cytology*, W. B. Saunders Co., London, 202.

28. SCHARFF, R., 1887, "On the Ovarian Eggs of some Osseous Fishes," *Quart. J. Micr. Sci.*, **28**.

29. SINGH, B. N. AND BOYLE, W., 1940, "The Vitellogenesis of *Gasterosteus aculeatus* (the Stickleback) investigated by the Ultacentrifuge," *Ibid.*, **81**.

30. SUBRAMANIAM, M. K. AND AIYER, R. G., 1935, " Oogenesis of *Acentrogobius neilli* with special reference to the behaviour of the nucleoli," *J. Roy. Micr. Soc.*, **55**.

31. SCHREINER, K. E., 1915, " Ueber Kern und Plasma Veranderungen in Fellzellen wahrend des Fettansatzes," *Anat. Anzeig.*

32. VAN DURME, M., 1914, " Nouvelles recherchs sur la vitellogeness des oeufs d' oiso-aux stades d' accroissement de maturation defecundation et du debut de la segmentation," *Arch. de Biol. tome*, **29**.

33. WALLACE, W., 1903, " Observations on Ovarian Ova and Follicles in certain Teleostean and Elasmobranch Fishes," *Quart. Journ. Micr. Sci.*, **47**, 161.

34. WILL, L., 1884, " Uber die Entsthung des Dotters, etc., Amphbien und Insecten," *Zoologischer Anzeiger. (Leipzig)*, **7**.

35. WILDON, E. B., 1928, *The Cell in Development and Heredity.*

EXPLANATION OF PLATE

FIG. 1. An oogonium showing a single nucleolus within the nucleus. *Notopterus*. Regaud-Tupa preparation stained with iron-alum hæmatoxylin, ×1330.

FIG. 2. An early oocyte of *Ophiocephalus* showing 8 nucleoli within the nucleus. Bouin preparation stained with iron-alum hæmatoxylin, ×630.

FIG. 3. Oocytes of *Clarias* showing a large number of nucleoli near the nuclear membrane. Bouin preparation stained with iron-alum hæmatoxylin, ×630.

FIG. 4. Photomicrograph of an young oocyte of *Ophiocephalus* showing many nucleoli within the nucleus, one extruded into the cytoplasm and one nucleolus just extruding out being situated between the nuclear membrane and the cytoplasm. Bouin preparation stained with Mann's methyl blue eosine.

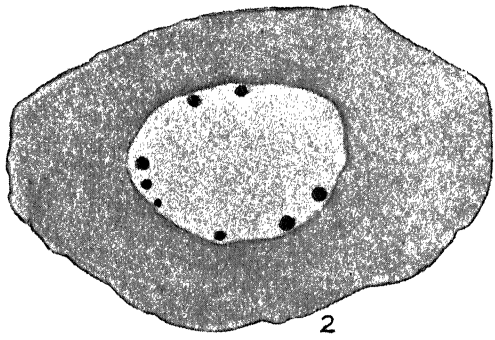
FIG. 5. An oocyte of *Ophiocephalus* showing two extruded nucleoli and one pointed nucleolus in the process of being extruded. Bouin preparation stained with iron-alum hæmatoxylin, ×630.

FIG. 6. An oocyte of *Rita* showing the nuclear membrane shrivelled a little and the nucleoli slightly separated from it, ×630.

LETTERING OF FIGURES

N, nucleus; *NU*, nucleolus; *NU. Ex.*, Nucleolar extrusion; *Nu. mem.*, Nuclear membrane.

1



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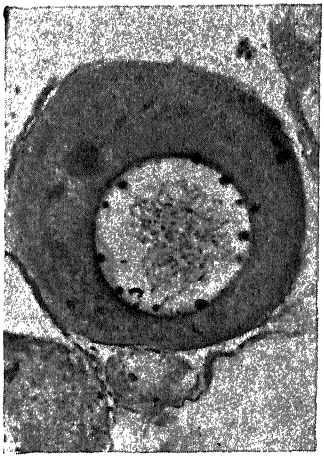

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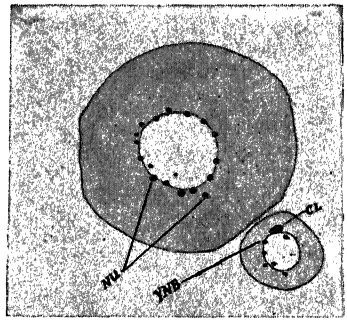

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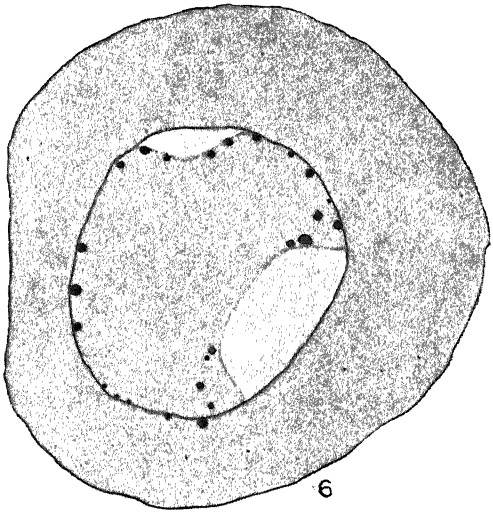

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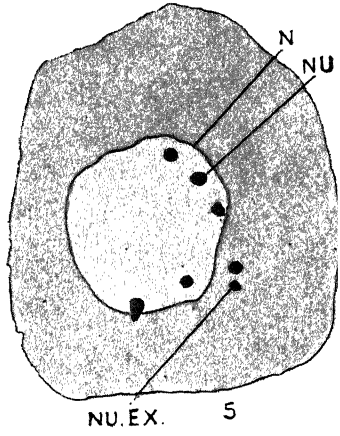

5

FIGS. 1–6

CYTOLOGY OF MILLETS

II. *Panicum* spp.

BY S. P. NAITHANI AND R. P. CHANDOLA

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ROOT tips were fixed in La Cour's 2 BE (1931). Flower buds for meiotic studies were fixed in Maeda's modification of Navashin's fluid (1934). All the material thus fixed was dehydrated, imbedded, sectioned and stained according to La Cour's schedule (1934).

OBSERVATIONS

Counting chromosomes at the polar view from the root tip cells revealed that *P. miliaceum*, *P. cruss-galli*, *P. colonum* and *P. ischane* had the diploid number as 36. *P. maximum* has $2n$–32 including two pairs of satellited chromosomes. This is in confirmation of the number reported by Moffet and Harcombe (1949).

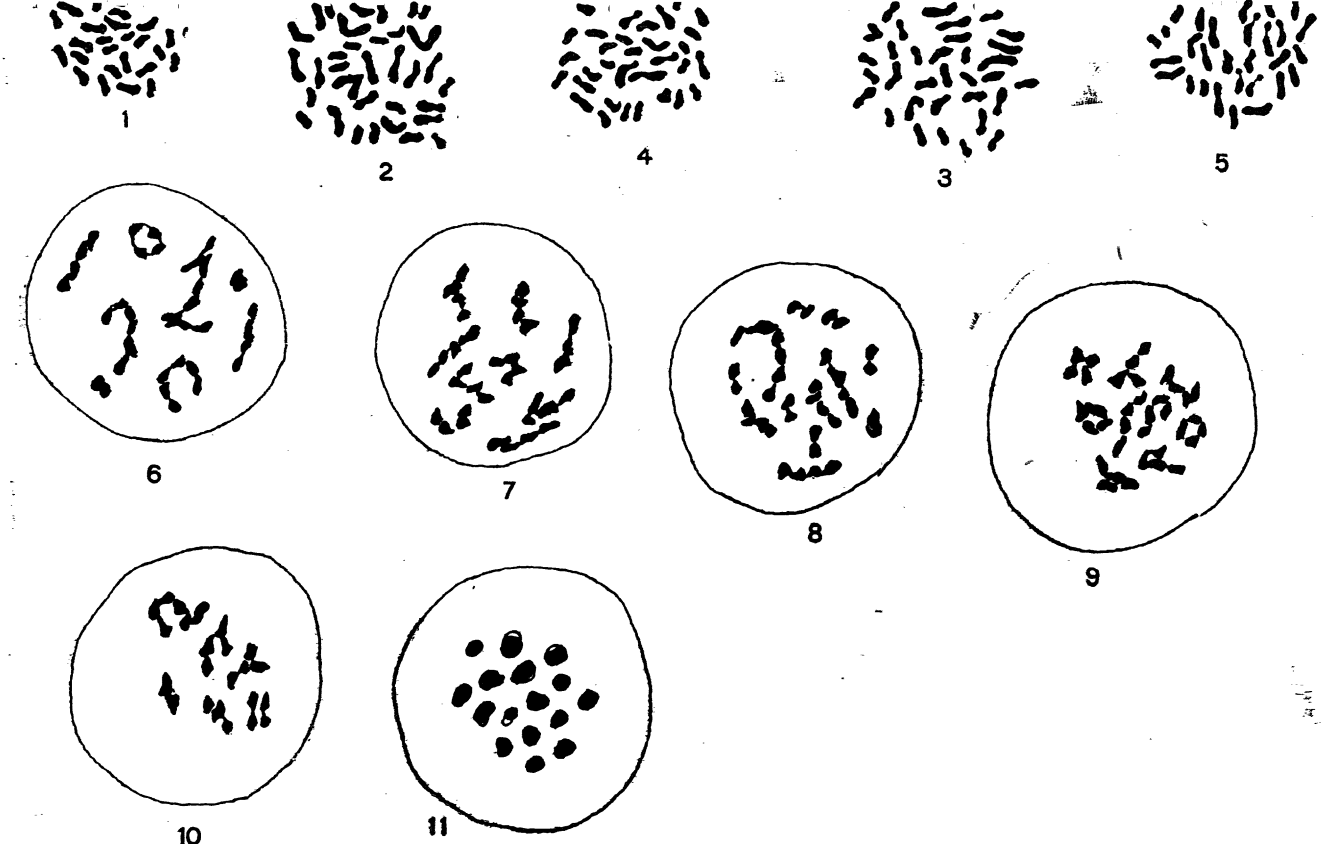
The meiotic studies showed that from earliest diakinesis multivalents association can be discerned. This association is variable in individual species due to differences of chiasma formation and development. In *Panicum miliaceum* counts made during diakinesis reveal in some cases much less than eighteen bodies. In some cases it was as low as 7 or 8. The average number seems to be between 10 and 12. Fig. 6 illustrates a cell at early metaphase I with 1_{viii}–2_{vi}–3_{iv}–2_{ii}. In some cases only quadrivalents were observed (Fig. 7). The following types of configurations are generally met with:—

TABLE I

Types of Configurations Observed at Metaphase I of P. miliaceum

viii	vi	iv	iii	ii	i
1	3	..	..	4	2
1	3	..	1	2	3
..	1	5	..	4	2
..	4	..	..	4	4
..	..	9	..	..	..
..	..	..	..	18	..

Pollen sterility is 32%.



FIGS 1–11.—Fig. 1. *Panicum ischane.* Somatic chromosomes (2*n*–36). Fig. 2. *Panicum miliaceum.* Somatic chromosomes (2*n*–36). Fig. 3. *P. cruss-galli.* Somatic chromosomes (2*n*–36). Fig. 4. *P. colonum.* Somatic chromosomes (2*n*–36). Fig. 5. *P. maximum.* Somatic chromosomes (2*n*–32). Fig. 6. Metaphase I of *P. miliaceum* with multivalents, bivalents and univalents. Fig. 7. *P. milliaceum* Metaphase I with quadrivalents only. Fig. 8. *P. cruss-galli.* Metaphase I with quadrivalents and multivalents. Fig. 9. *P. ischane.* Metaphase I with quadrivalents only. Fig. 10. *P. maximum* Metaphase I with multivalents, quadrivalents and bivalents. Fig. 11. *P. maximum:* Polar view of Metaphase I with sixteen bivalents.

At diakinesis of *P. cruss-galli* multivalent formations of chromosomes is prevalent though to a lesser extent (Fig. 8). The types of these associations are summarised in Table II.

TABLE II

Frequency of Multivalent Formation in P. cruss-galli *at Metaphase I*

vi	ii	i
8	2	..
9	..	..
6	4	4
4	8	4
4	10	..

In *Panicum colonum* late diakinesis plates show many multivalents. In the 30 nuclei examined quadrivalents, bivalents and univalents were observed. No hexa- or octavalents were observed. Pollen sterility is 45%. Metaphase I plates in *P. ischane* also show multivalent formation and the pollen counts confirm that meiotic irregularity is the cause of pollen sterility here also. Pollen sterility is 35% (Fig. 9). In *P. maximum* metaphase I plates present both multivalents as well as bivalents (Fig. 10). Sixteen bivalents were, however, ascertained in many instances (Fig. 11).

By studying the pollen sterility, fertility and seed germination rate, we come to the conclusion that meiotic irregularity is the sole cause of low rates of fertility, germination and setting of seeds.

DISCUSSIONS

Chromosome Pairing at Meiosis in genus Panicum

In the genus *Panicum* chromosome pairing at meiosis reveals that autopolyploidy has played a major role in evolution. Muntzing (1936) and Neilsen (1944) also come to this conclusion. In this genus multivalents involving as many as eight chromosomes are frequently met with. This is perhaps due to non-homologous chromosomes taking part in forming such associations. Besides homologous pairing which is subject to so much variations, non-homologous pairing also can take place under certain circumstances. Yarnell (1931) noticed that in triploid *Fragaria* with 21 chromosomes there were three associations of four chromosomes, one triivalent

and three pairs. Longely (1924) notices similar condition in *Rubus* and *Citrus*. McClintock (1934) found in *Zea mays* an association of non-homologous segment in cytologically unbalanced forms. In this case the prophase association of non-homologous pairing was as intimate as homologous one. Lammerts (1934) not only finds non-homologous parts paired at pachytene but adduces evidence in favour of the view that such pairing can and often does persist until metaphase due to the formation of chiasmata. We, however, think that in these and any other such cases interchanges in chromosome segments rather than non-homologous pairing is responsible for multivalent formation in polyploids. Also in *Panicum* autopolyploids and upset in genic balance is due to chromosomes being duplicated. Randolf (1941) also recognised that meiotic irregularities cause sterility and agreed that genic disturbance is the main cause.

LITERATURE CITED

LA COUR, L., 1931, "Improvement in everyday technique in plant cytology," *J. R. Micro. Soc.*, **51**, 119–26.

LAMMERTS, 1934, "On the nature of chromosome association in *Nicotiana tabacum* haploids," *Cytologia*, **6**, 38–50.

LONGELY, A. E., 1924, "Triploid Citrus," *J. Wash. Acad. Sci.*, **16**, 455–89.

MOFFET, A. S. AND HARCOMBE, R., 1949, "Chromosome numbers in South African grasses," *Heredity*, **3**, 109–12.

MUNTZING, A., 1933, "The evolutionary significance of autopolyploidy," *Heriditas*, **21**, 263–378.

NAVASHIN, M., 1934, "Chromosome alterations caused by hybridization and their bearing upon certain genetical problems," *Cytologia*, **5**, 169–203.

NEILSEN, E. L., 1944, "Analysis of variation in *Panicum vergatum*," *J. Ag. Res.*, **69**, 327–53.

YARNELL, S. H., 1931, "A study of certain polyploid and aneuploid forms of *Eragaria*," *Genetics*, **16**, 455–89.

RANDOLF, L. F., 1941, "An evaluation of induced polyploidy as method of breeding crop plants," *Am. Nat.*, **75**, 347–63.

ROLE OF SOME AMINO ACIDS ON THE GROWTH OF *PHYLLOSTICTA CYCADINA* (PASSER) AND *PESTALOTIA MANGIFERA* (BUTL.)

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Amino acids serve as good source of nitrogen. They are present in free form in the plants and are also obtained by the hydrolysis of the protein complex. From a review of the literature it appears that amino acids are assimilated by a large number of fungi. Koltz (1923) showed that *Aspergillus niger* and *Diploidia natalensis* could use amino acids well. Wolf and Shoup (1943) working on *Allomyces arbuscula* observed that alanine, aspartic acid, asparagine, arginine, cystine, glutamine acid and leucine served as a good source of nitrogen. They also found that *A. javanicus* utilized aspartic acid, asparagine, cystine and glutamic acid, whereas *A. moniliformis* and *A. cystogenus* utilized only alanine, aspartic acid and glutamic acid. None of these species were able to use glycine and tyrosine. Tandon (1950) observed that *Pestalotia malorum* and *P. psidii* responded favourably when glycine and tyrosine were used as a source of nitrogen.

A review of the existing literature clearly establishes that there is a marked specificity amongst the micro-organisms for the kind of nitrogen they required in their metabolism. An attempt has, therefore, been made to study the amino acid requirement of *Phyllosticta cycadina* and *Pestalotia mangifera*.

Material and Methods

The organisms were isolated from the infected leaves of *Cycas revoluta* and *Mangifera indica* respectively.

Asthana and Hawker's medium A* which has potassium nitrate as the source of nitrogen was selected as the basal medium. The amount of nitrogen in KNO_3 was calculated and it was replaced by equal quantity of nitrogen in various amino acids.

Throughout this work Pyrex glasswares and purest available chemicals were used. 25 c.c. of nutrient solution was placed in 150 c.c. Erlenmeyer flasks and sterilized in an autoclave at 15 lb. pressure for 15 mts. For each

* KNO_3 3·5 gm., Glucose 5 gm., KH_2PO_4 1·75 gm., $MgSO_4 7H_2O$/0·75 gm. and distilled water 1 litre.

series four replicates were used and inoculations were made by agar disc method. The organism was allowed to grow for 15 days. At the end of this period the fungal mats of each flask were collected separately on previously dried and weighed Whatman's filter-paper No. 42. The fungus was dried in electric oven at 65° C. and was transferred to a desiccator before weighing it quickly. This constant weight which was mostly obtained after 3 days was used as the quantitative measure for the growth of the fungus.

Following 11 amino acids were tried:—

Phenyl alanine, glycine, *dl*-valine, glutamic acid, serine, tyrosine, histidine, leucine, aspartic acid, alanine and arginine.

Amino acids (both free and those obtained by hydrolysis of protein complex) present in the leaves of *Cycas revoluta* and *Mangifera indica* were also found by circular paper chromatography and the following results were obtained.

In Cycas revoluta *leaves*

(*a*) *Free Amino acids*	(*b*) *Amino acids obtained by hydrolysis of protein complex*
1. *dl*-Valine	1. *dl*-Valine
2. Glutamic acid	2. Tyrosine
3. Aspartic acid	3. Glycine
4. Alanine	4. Glutamic acid
5. Histidine	5. Arginine
6. Arginine	6. Histidine
7. Tyrosine	

In Mangifera indica *leaves*

(*a*) *Free Amino acids*	(*b*) *Amino acids obtained by hydrolysis of protein complex*
1. Aspartic acid	1. Histidine
2. Glutamic acid	2. Arginine
3. Tyrosine	3. Aspartic acid
	4. Glutamic acid
	5. Alanine
	6. *dl*-Tyrosine
	7. *dl*-Valine
	8. Leucine

OBSERVATIONS

In Phyllosticta cycadina

Both macroscopic and microscopic characters were recorded.

Macroscopic Characters.—It was observed that growth was uniform on all the amino acids tried in the present investigations except in phenyle alanine, glycine, serine and leucine where it was patchy. The thickness of the mat in each case varied considerably.

Microscopic Characters.—The microscopic characters were also similar on all the media except for a slight change in the thickness of the mycelium or in the size of chlamydospores or pycnidia. Mycelium was thin, septate with granular protoplasm. Chlomydospores were mostly in chains. Pycnidia were of dark brown colour with reticulate thickenings on their walls. The size of the ostioles was generally proportional to the size of the pycnidia. Spores were small oval and hyaline of uniform size.

In Pestalotia mangifera

Macroscopic Characters.—The growth was uniform on all the media; but there was marked difference in the thickness of the mats. The organism secreted black drops which were more numerous on media containing phenyl alanine and glutamic acid and were absent on arginine.

Microscopic Characters.—The hyphæ were thin white, septate, branched with granular protoplasm. Chlamydospores which were mostly in chains were obtained on the media containing glycine, phenyl alanine, histidine and serine as the source of nitrogen. Spores were mostly 5-celled. The three central cells were deeply coloured, while the two apical cells were hyaline with a beak on one side and 2 or 3 cilia on the other side. The length of the cilia ranged between 9–13 μ. Sometimes the spores were 1 or 4 or 6-celled but the number of 5-celled spores dominated in each case.

The dry weight, sporulation and spore size of the two organisms are recorded in Table I.

From Table I it is evident that *Phyllosticta cycadina* showed good growth when *dl*-valine, glutamic acid, aspartic acid or alanine were used as the source of nitrogen. Phenyl alanine, glycine, serine and leucine were poor sources of nitrogen. Intermediate results were obtained on tyrosine, histidine and arganine.

Pestalotia mangifera showed best growth on media containing tyrosine, *dl*-valine, leucine and glutamic acid. Glycine was the poorest source of nitrogen.

TABLE I

Showing dry weight and other microscopic characters of Phyllosticta cycadina *and* Pestalotia mangifera *on different amino acids*

Amino acids	Phyllosticta cycadina			Pestalotia mangifera		
	Dry weight in mgm.	Sporulation	Spore size in μ	Dry weight in mgm.	Sporulation	Spore size in μ
Phenyl alanine	51·4	Poor	3·5×1·5	63·6	Good	21·6×7·0
Glycine ..	52·5	Poor	3·6×1·3	37·0	Absent	
dl-Valine ..	124·9	Fair	3·3×1·3	119·0	Fair	24·6×6·6
Glutamic acid ..	117·4	Poor	3·9×1·3	104·2	Good	19·9×5·9
Serine ..	46·0	Poor	3·4×1·4	63·0	Absent	
Tyrosine ..	81·4	Fair	3·3×1·5	200·0	Fair	21·6×6·9
Histidine ..	93·5	Good	3·9×1·5	60·0	Poor	21·3×6·6
Leucine ..	42·5	Poor	3·9×1·6	113·0	Poor	22·6×5·6
Aspartic acid ..	108·8	Fair	3·9×1·6	98·4	Fair	21·6×6·9
Alanine ..	102·0	Good	3·6×1·5	72·3	Fair	21·3×6·3
Arginine ..	82·4	Fair	3·9×1·3	59·4	Fair	22·6×6·3

Phyllosticta cycadina showed best sporulation on histidine and alanine while *Pestalotia mangifera* showed best sporulation on phenyl alanine and glutamic acid. *dl*-Valine, tyrosine, arginine and aspartic acid induced moderate sporulation in both the organisms. Sporulation of *Phyllosticta cycadina* was poor on leucine, serine, glutamic acid and glycine. *Pestalotia mangifera* showed poor sporulation on histidine and leucine. This organism did not sporulate on serine or glycine.

The spore size of both the organisms was fairly constant on different amino acids. The colour of the spores of *Pestalotia mangifera* was slightly influenced by the nature of the nitrogen compound available. It was olive brown on phenyl alanine, buffy brown on tyrosine and deep olive on aspartic acid.

DISCUSSION

The importance of amino acids in the nutrition of fungi lies in the fact that they are present in free form in the plants and so fungi can easily utilize them.

In the present study *dl*-valine, glutamic acid, aspartic acid and alanine were found to be the best amino acids for the growth of *Phyllosticta cycadina.* Wolf (1953), Srivastava (1951) and Singh (1954) also found that *dl*-valine and alanine were best for the growth of *Ustilago zeæ, Curvularia lunata* and *Glœosporium musarum* respectively. Steinberg (1942) working on *Aspergillus niger* and Brock (1951) on *Morcella esculenta* reported *dl*-valine to be a poor source of nitrogen, for the growth of their fungi. Steinberg (1942) had found that glutamic acid and aspartic acid were good sources of nitrogen for *A. niger.* The presence of all these four amino acids in the leaves of *Cycas revoluta* may be significant as they are all good for the growth of this fungus.

Wolf and Shoup (1943) obtained no growth of a number of species of *Allomyces* on glycine but in the present investigation, glycine supported some growth. Leucine, serine, phenyl alanine supported more or less similar growth.

Tyrosine supported best growth of *Pestalotia mangifera* but it was not so suitable for *Phyllosticta cycadina.*

It is interesting to observe that glutamic acid and *dl*-valine are suitable for the growth of both the organisms.

Sporulation of *Phyllosticta cycadina* is best on histidine and alanine while that of *Pestalotia mangifera* is good on glutamic acid and phenyl alanine. These results not only indicate that the different organisms sporulate better on different substances but they also establish that the best growth may not be associated with best sporulation.

Summary

Phyllosticta cycadina and *Pestalotia mangifera* were grown on a number of amino acids. It was observed that aspartic acid, alanine, glutamic acid and *dl*-valine supported good growth of *Phyllosticta cycadina* while leucine, tyrosine, *dl*-valine and glutamic acid gave best growth of *Pestalotia mangifera.* Serine, glycine, phenyl alanine did not support good growth for both the organisms. *Phyllosticta cycadina* sporulated best when grown on histidine or alanine, while glutamic acid and phenyl alanine induced best sporulation of *Pestalotia mangifera.*

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References

1. Brock, T. D., 1951, "Studies on the nutrition of *Morchella esculenta*," *Mycologia*, **43**, 402–22.

2. STEINBERG, R. A., 1942, " Effect of trace elements on the growth of *Aspergillus niger* with amino acids," *Jour. Agri. Res.*, **64**, 455–75.
3. SRIVASTAVA, J. P., 1951, " Studies of *Curvularia lunata* (Wakker)," *Proc. Nat. Acad. Sci.* (*India*), **21 B**, 117–37.
4. SINGH, J., 1954, *D' Phil. Thesis*, University of Allahabad.
5. TANDON, M. P., 1950, *Ibid.*
6. WOLF, F. T., 1953, " The utilization of nitrogen and carbon compounds by *Ustilago zeæ*," *Mycologia*, **45**, 516–22.
7. WOLF, F. T. AND C. S. SHOUP, 1943, The effect of certain sugars and aminoacids upon despiration of *Allomyces*" *Mycologia*, **35**, 192–200.

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